

REMARKS

Claims 26-32, 34, 36-41, 46, and 52-57 are currently pending in this application.

All of these claims stand rejected for lack of enablement. 35 U.S.C. § 112, 1st ¶. The sole question remains to be whether Heinen¹ shows that the claimed vaccines exacerbate flu symptoms in pigs, and therefore casts doubt on the efficacy of the claimed vaccines.

The Examiner states that she is not persuaded by applicants' arguments submitted in their previous response (October 15, 2004 Response to July 15, 2003 Office Action). The Examiner's remarks suggest that there is confusion concerning applicants' arguments.

A. An Plasmid Vector Is Not a Proper Control for a Protein Vaccine

The Examiner states that Heinen's empty plasmid was a proper negative control for the M2e protein vaccine experiment, because the plasmid did not induce immune responses as assessed by antibody titers and lymphoproliferative responses of PBMCs. Office Action, pp. 2 and 3. Applicants respectfully traverse.

Heinen examined IgG titers and T lymphocyte proliferation in animals treated with an M2e vaccine or with a "control." See Figs. 3 and 4. However, IgG titers and T lymphocyte proliferation are only two of the many components of an immune response, a highly complex process. For example, Heinen did not measure the titers of other antibody

¹ Heinen et al., Journal of General Virology 83:1851-9 (2002).

isotypes, e.g., IgM, IgD, and IgA. It is unclear whether or not the empty plasmid elicited any change in the immune system with regard to the untested components.²

Further, the plasmid control was injected **intradermally**, whereas the protein vaccines were injected **intramuscularly**. The Examiner states that this difference is of no issue because Figs. 3 and 4 show that the plasmid control elicited no immune response. The Examiner is mistaken. Due to this very difference in the route of administration, it is not clear whether the plasmid control would have elicited an immune response in the test animal if it had been injected **intramuscularly**, as it should have been, like the protein vaccines.

In sum, Heinen's empty plasmid, due to both its form and route of administration, was not a proper negative control for the protein vaccine.

B. Heinen's Negative Control Caused the Highest Fever

The Examiner states that "[w]hile the negative control group developed the highest fever on most days, the fevers the experimental groups developed closely shadow the negative control group and are not significantly different." Office Action, pp. 3 and 4. That is not true. The difference between the experimental groups and the control group was statistically significant.

Fig. 2B shows the temperatures of the various groups of test animals on different days post challenge. Relevant here are group 1, pigs immunized with M2eHBc, indicated by solid triangle; group 2, pigs immunized with M2eHBc plus adjuvant, indicated by solid

² See also applicants' previous response at p. 12, first ¶.

diamond; and group 4, pigs immunized with the empty plasmid, indicated by open square. The legend of Fig. 2B explains:

Significant differences ($P < 0.05$) of the mean in the M2eHBc fusion protein (a), M2eHBc protein + adjuvant (b) and M2eNP DNA (c) immunized groups are indicated. (emphasis added)

In other words, where there is a statistically significant difference between group 1 (M2eHBc alone; solid triangle) and group 4 ([empty plasmid;] open square), the data point is indicated by an “a” on top of the graph. Where there is a statistically significant difference between group 2 (M2eHBc plus adjuvant; solid diamond) and group 4, the data point is indicated by a “b.”

Accordingly, Fig. 2B shows that the mean body temperature of group 4, the so-called negative control group, was **statistically** higher than that of group 2 (M2eHBc plus adjuvant) on days 1.5, 2, 5, and 7. The mean body temperature of this “control” group was also **statistically** higher than that of group 1 (M2eHBc alone) on day 7. Thus, Heinen’s own data, even if taken at their face value, would suggest that M2eHBc immunity reduced fevers in swine.

C. Heinen’s “Clinical Signs” Score Is Highly Subjective

The Examiner states that Heinen’s evaluation of coughing is not subjective and therefore, Heinen’s use of “clinical signs” is proper. Applicants agree that coughing is an objective symptom of flu. See applicants’ previous response at pp. 14 and 15. However, by concluding that Heinen’s use of clinical signs is therefore proper, the Examiner appears to have equated Heinen’s “clinical signs” with coughing. That is incorrect.

As applicants explained in the previous response, Heinen's "clinical signs," which is scored in Fig. 2A, actually include **five** signs: labored breathing, abdominal breathing, anorexia, apathy and coughing (p. 1854, left col., last full paragraph). Of those five signs, only coughing can be considered as a reliable and objective indicator of flu infection.³ But Fig. 2A **does not** just score coughing. Fig. 2A shows the **combined scores** of the five signs, four of them are highly subjective and have not been established in the art as reliable indicators for flu.⁴ Thus, the combined scores shown in Fig. 2A are not creditable.⁵

Last but not least, the authors do not indicate that the measurements of these "clinical signs" were done in a blinded manner. This deficiency also undercuts the credibility of Heinen's "clinical signs" data.

D. Heinen Fails to Prove Its Conclusions

Due to the various deficiencies in the design of the experiments, Heinen's data fail to provide any credible evidence on whether or not the claimed vaccines are efficacious. This paper at best shows that a **human** M2e vaccine does not protect pigs challenged with a **swine** influenza virus. However, this is not pertinent to the pending claims, as the claims as

³ See Monto, a copy of which is enclosed with a Supplemental Information Disclosure Statement filed herewith.

⁴ See Monto.

⁵ The Examiner also raises issues with respect to whether the claimed vaccine provides heterologous protection. However, this issue is not relevant because the claims do not require heterologous protection.

last amended require that the M2e portion of the claimed vaccine be from the same species of animal for which the vaccine is intended.⁶

E. New Studies by Others Demonstrate the Efficacy of the Claimed Vaccines

The efficacy of M2e vaccines has been supported by recent independent studies.

Applicants submit Fan, a newly published study from a group of Merck scientists demonstrating that vaccines that fall with the scope of the pending claims are efficacious in animals other than mice.⁷

Fan's vaccines are M2e conjugated to a carrier that is either keyhole limpet hemocyanin (KLH) or *Neisseria meningitidis* outer membrane protein complex (OMPC).

Fan's experiments demonstrate that

The conjugate vaccines were highly immunogenic in **all** species tested and were able to confer both protection against lethal challenge of either H1N1 or H3N1 virus in mice and reduce viral shedding in the lower respiratory tracts of **mice** and **ferrets**. The protection against lethal challenge in mice could also be achieved by passive transfer of **monkey** sera containing high M2 antibody titers. (Abstract; emphasis added)

In contrast to Heinen, Fan's experiments used proper controls. The control groups were immunized with adjuvant only, using the same regimen. See, e.g., p. 2996, right col., last full paragraph, lines 9 and 10; p. 2997, Fig. 2, lines 5 and 6.

⁶ The Examiner states that the M2e DNA vaccine also exacerbates flu symptoms in pigs. Those data are not relevant to the current discussion because the pending claims are not directed to DNA vaccines. Applicants reserve the right to address those data when pursuing claims to DNA vaccines.

⁷ Fan et al., "Preclinical study of influenza virus A M2 peptide conjugate vaccines in mice, ferrets, and rhesus monkeys," *Vaccine* 22:2993-3003 (March 2004). This paper is enclosed with the Supplemental Information Disclosure Statement filed herewith.

Fan's data confirm the efficacy of applicants' claimed vaccines. Fan states:

. . . Indeed, we have shown in the present study that M2 peptide conjugate vaccines prepared from both KLH and OMPC carriers are highly immunogenic in **mice, ferrets, and monkeys**. More importantly, the antibody responses raised by these vaccines are **protective** against live influenza virus challenge. The results are consistent with more recent reports by Mozdzanowska et al. and by Liu et al. in that the former showed that M2 multiple antigenic peptides were able to induce protection in **mouse** challenge model, whereas the latter demonstrated that M2 peptide KLH conjugates were able to elicit **rabbit** antibody response containing in vitro viral inhibitory activities. . . .

We found M2-OMPC conjugate vaccine to be rather **potent in monkeys**. . . . (p. 3001; emphasis added)

These statements echo the huge body of literature validating the use of mouse models for flu vaccine development.⁸ These statements also confirm that the M2e vaccines are useful in animals other than mice. The authors conclude:

The data reported here support the concept of developing an M2 peptide-OMPC carrier conjugate vaccine that can be used as an adjunct to the current vaccine products for elicitation of broadly-reactive and clinically protective anti-influenza virus A antibodies. Such a vaccine would not have to be updated annually and could serve as a possible pediatric vaccine that could provide early protection against influenza virus-mediated disease. (p. 3002, last ¶)

Fan publishes two years after Heinen, and is a reflection of the current state of the art. Fan shows that the current state of the art confirms that applicants' invention works for its intended purpose and is enabled.

⁸ See, e.g., applicants' November 4, 2002 Response to the July 3, 2002 Final Office Action, p. 7.

CONCLUSION

Applicants respectfully submit that the application is in condition for allowance.

To expedite prosecution, the Examiner is invited to telephone the undersigned to discuss any issues remaining in this application.

Respectfully submitted,



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